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Thesis

NEWER FUNGICIDAL AGENTS

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SECTION A

A DISCUSSION OF THE NEWER FUNGICIDAL AGENTS

FOREWORD

The problems involved in the treatment of superficial fungous infections are manifold. The clinician recognizes the need of coping with the infection and re-establishing the growth of tissue in the infected area; the mycologist is concerned with the etiologic agent responsible for the condition, while the pharmacist observes the problem as one of properly dispensing the medication to be used in combatting the infection.

To one who is a mixture of both pharmacist and mycologist, an investigation of the agents used in the treatment of various superficial mycoses seems most timely.

This investigator then was motivated from a dual standpoint. It is the purpose of this thesis, to present a survey of the latest agents used in the control and treatment of certain of the fungous infections of mankind. While the main theme of this study will deal with fungicidal agents, a considerable portion of it will deal with a discussion of the *in vitro* evaluation of these agents and various factors that may modify their activity.

It is hoped that this study will represent a worthwhile contribution to the science of medical mycology.

GENERAL HISTORY

As far back as 1677, Hooke discovered that fungi belonging to the genus Phragmidium were responsible for the yellow spots on roses. The first fungous infection of human was demonstrated by Langenbeck (1839) who found that thrush, a condition characterized by the presence of soft whitish patches on areas rich in mucous membrane, was due to a yeast-like organism. That same year, Schoenlein described a long, narrow, ribbon-like organism as the causative agent of favus or honeycomb ringworm. Description of the fungi causing tinea circinata, the classic form of ringworm, and tinea versicolor, characterized by yellow or brownish patches on the skin, followed in 1842 and 1846. In 1870, Tilbury Fox described tinea pedis which will be discussed in detail later in this report.

Interest in the fungous diseases of man declined from the middle of the nineteenth century until the publication of Les Teignes (1910) by Sabouraud. This book, which was well written and beautifully illustrated, brought mycology back to the attention of physicians when it had been almost forgotten. Other workers, notably Williams who became interested in the dermatoses of the hands and feet, Hopkins with his contributions on

moniliasis and ringworm, Weidman who did extensive laboratory studies, Sulzberger who contributed a great deal on dermatologic allergy, and Peck and Lewis for their contributions on the Trichophytons, helped to revive interest in the subject.

The problem of superficial mycoses and their treatment is such an extensive one that this study was limited to two of the most common conditions afflicting mankind, namely, athlete's foot and ringworm of the scalp. These will be discussed separately.

HISTORY OF TREATMENT

The treatment of superficial fungous infections, even as early as 1880, consisted of topical applications of antiseptics such as iodine and phenol and the internal administration of tonics such as sulfur and vegetable drugs. In 1883, Duhring used mercurials, sulfur, creosote, phenol and iodine to kill invading fungi. He recognized acute and chronic types of fungous infection and stated that either condition should be treated symptomatically.

Tinea pedis

During recent years, fungous infection of the feet (athlete's foot) has attracted increasing attention. In fact, it has been called a disease due to civilization and it is estimated that some 75 per cent of the population is infected at one time or another with this condition. The infection generally manifests itself between the toes or on the sole of the foot. It is accompanied by a great deal of itching and discomfort and may spread to other parts of the body.

The causative agent for this condition is an organism belonging to the Fungi Imperfecti. More specifically, it can be classified as follows:

Order - Conidiosporales

Suborder - Aleuriosporineae

Genus - Trichophyton

Species - Trichophyton mentagrophytes

It should be pointed out that the species Trichophyton mentagrophytes includes many varieties such as Trochophyton gypseum, Trichophyton interdigitale, and Tinea pedis. Macroscopically, this organism appears as a white fluffy growth which becomes velvety and buff colored after about two weeks of growth on artificial culture media. On microscopic examination, it exhibits a large number of filamentous hyphae and various types of spores with an abundance of the aleuriospores (see Fig. 1, page 6).

Various medications have been used to combat this condition. Schamberg (1917) used ointments and lotions to treat this condition. He also advocated careful cleansing of the affected areas, debridement of dead skin and wet dressings to allay inflammation. His medications consisted of dilute tincture of iodine, betanaphthol, resorcin, tar, ammoniated mercury and chrysarobin. He also used Whitfield's ointment, a mixture of salicylic and benzoic acids, as a keratolytic agent. In 1927, Sutton advocated Whitfield's ointment as a specific for this condition. He,too, debrided dead skin and in acutely

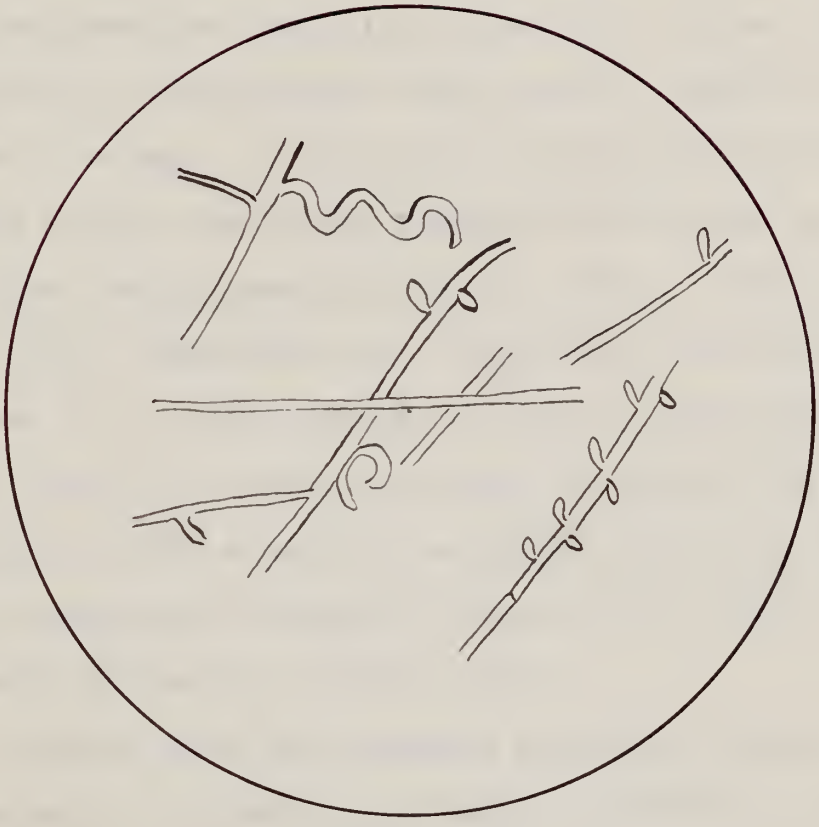

Fig. 1 - Trichophyton mentagrophytes
Note the spiral hyphae and
aleuriospores.

inflamed conditions used soothing remedies such as Burow's solution(a solution of aluminum subacetate).Sutton also mentioned the use of mercurochrome, methylene blue, picric acid and potassium permanganate. In 1935, Woodward and others found that the introduction of halogen atoms into dihydric phenols increased their fungicidal powers. They also found that the fungicidal powers of iodine, hexylresorcinol and chlorothymol were greatly reduced in the presence of serum. In 1938, Peck and Rosenfeld called attention to the fungicidal powers of the fatty acids and their normal salts contained in the sweat. Peck and others (1939) stated that the fungicidal properties of sweat were due to its content of acetic, propionic, caproic, caprylic, lactic and ascorbic acids. The two publications by Peck and his co-workers were the basis for the tremendous attention focused on the salts of the fatty acids during the ensuing years.

In 1943, Peck and Schwartz presented formulae for the treatment of superficial fungous infections. These formulae will be discussed later in this study.

Following the brilliant contributions of Peck and his associates, other workers, notably Keeney, Hopkins, Goldman, Shapiro and Rothman substantiated their findings and laid the foundation for a series of therapeutic agents

that represent a safe and physiological approach in the treatment of tinea pedis as well as many other fungous infections.

Tinea capitis

Tinea capitis or ringworm of the scalp is a highly contagious fungous infection occurring in children below the age of puberty. The sources of infection fall into two main groups. The first, or so-called "animal" type of infection is caused by Microsporum lanosum. The second type of infection, called the "human" type, is caused by Microsporum audouini. The symptoms caused by these organisms are somewhat similar although the latter type of infection is more persistent and harder to overcome. In both cases there are one or several scaling, inflammatory patches on the scalp. Pustules occur around the hair follicles and the hair may fall out leaving bald patches on the scalp.

Both of the causative organisms belong to the Fungi Imperfecti and may be further classified as follows:

Order - Conidiosporales

Suborder - Aleuriosporineae

Genus - Microsporum

Species - Microsporum { lanosum / audouini }

Morphologically Microsporum lanosum appears as a

downy growth which develops fairly rapidly. After two weeks the colony becomes woolly and the aerial material abundant. Microscopically, the outstanding characteristic is the presence of a large number of fuseaux (spindle-shaped spores) of a tapering sort. Microconidia and some other forms of spores may be noted(see Fig. 2, page10).

Microsporum audouini colonies first appear as a cottony elevation which becomes grayish-white after about two weeks. Aerial growth is sparse. Microscopically, there are some fuseaux but they are fewer in number than those seen in M. lanosum. Hyphae are abundant(see Fig. 3, page11).

In the treatment of this, we find a wide variation in the types of medication used throughout the last seventy years. In 1883, Duhring advised manual epilation of diseased hairs and various topical applications. He painted chronic patches with glacial acetic acid or with cantharides in collodion. He also used sulfur, ammoniated mercury, creosote, phenol, mercuric nitrate and iodine as daily applications.

Corlett (1899) also advocated manual epilation and phenol 4-6 percent, copper oleate and 20 per cent vaseline in formalin.

In 1917, Schamberg used betanaphthol 12 per cent, tar 35-50 per cent, chrysarobin 4 - 7 per cent, as well

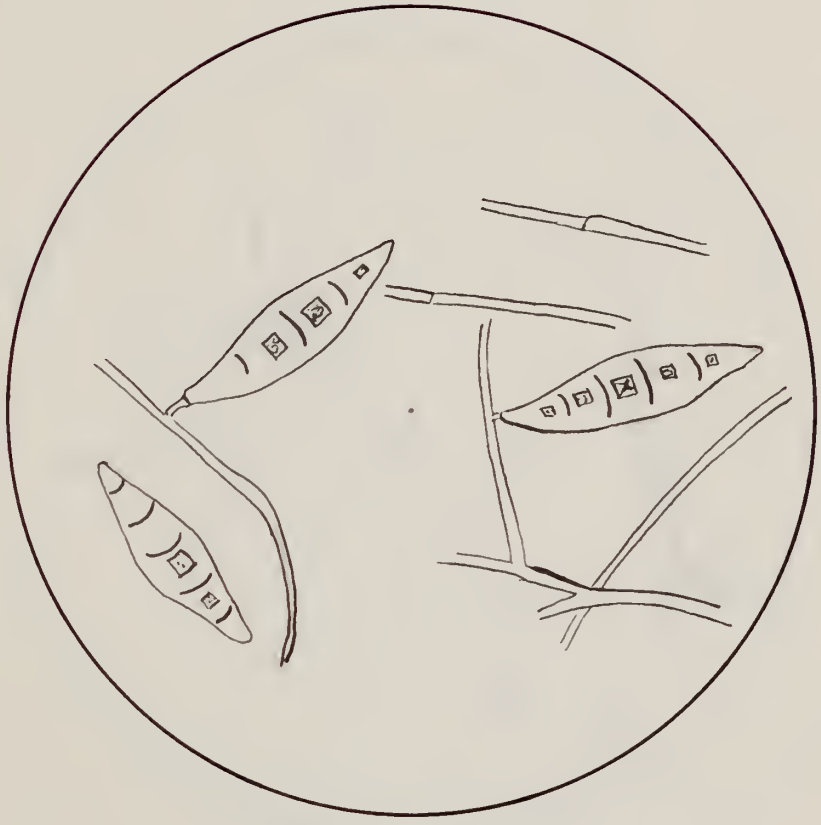

Fig. 2 - Microsporum lanosum - cause of "animal type" of tinea capitis. Note the spindle-shaped spores (fuseaux).

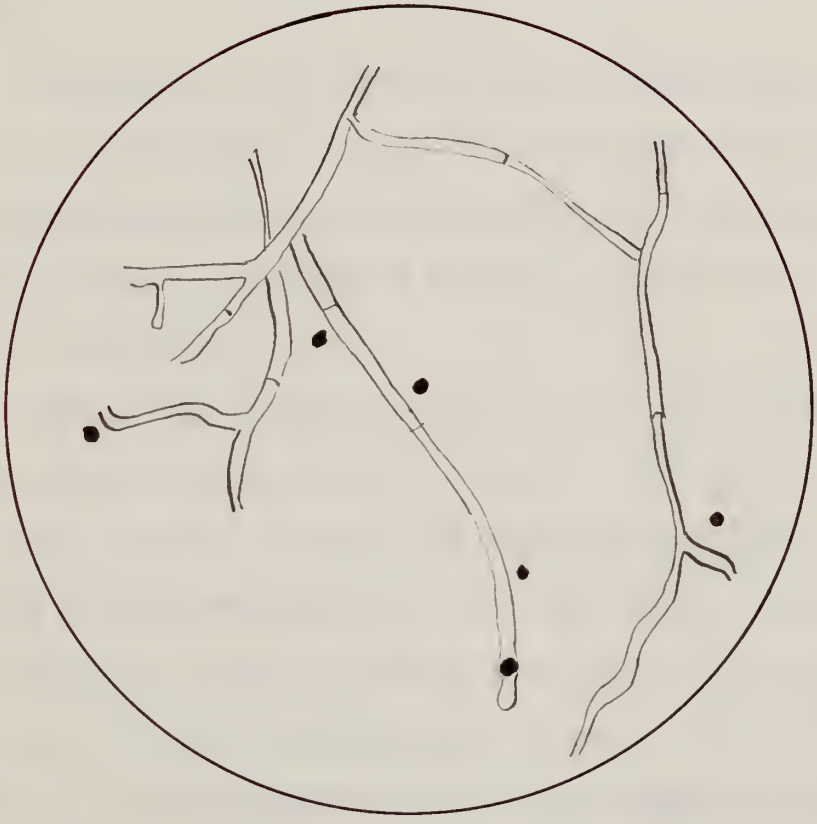

Fig. 3 - Microsporum audouini - cause of "human type" of tinea capitis.

as manual epilation.

Darier (1920) painted tincture of iodine over the scalp and applied iodized vaseline or chrysarobin ointment.

In 1927, Sutton pointed out that iodine, ammoniated mercury, chrysarobin, sulfur, tar and betanaphthol were the best fungicidal agents for the treatment of this condition.

Livingood and Pillsbury observed 105 cases of tinea capitis and noticed no consistent good effect from any of the following fungicides: tincture of iodine, phenyl mercuric nitrate, ammoniated mercury, sulfur and salicylic acid(1941).

Perhaps the greatest advance made in the treatment of this condition was made by Schwartz and his associates of the United States Public Health Service. These workers undertook an investigation of over 600 cases at Hagerstown, Maryland. They evaluated seventeen different topical applications during their survey, including the following: zinc undecylenate and undecylenic acid, sodium propionate and propionic acid, copper oleate, colloidal iodine, a number of quaternary ammonium compounds, pentachlorphenol, phenyl mercuric salicylate, copper undecylenate and salicylanilide. From this objective study, these workers recommend copper undecylenate and salicylanilide as the most satisfactory agents for the treatment of tinea capitis.

AGENTS USED IN THE TREATMENT OF TINEA PEDIS

General Discussion

Before discussing the various agents used in the treatment of superficial fungous infections of any sort, it would seem logical to enumerate the attributes that should be possessed by such a compound. They are as follows:

1. It should be able to kill the particular fungus or fungi on contact.
2. It should be able to penetrate to the location of the infection.
3. It should be non-irritating and non-toxic.
4. It should have bactericidal as well as fungicidal action.

It is startling to learn of the enormous number of preparations and concoctions that have been advocated and used in the treatment of this condition. Underwood (1946) listed over one hundred different preparations that ranged all the way from mixtures that were soundly prescribed to compounds that verged on sheer quackery. In his classification, he listed powders, ointments, tinctures and lotions and stated that, "The human foot has become the target for unbelievable chemical abuse." While the ensuing discussion cannot hope to describe all of the preparations reported by Underwood, the most

commonly used groups of agents will be discussed and evaluated.

Keratolytics

Keratolytics are agents that are able to dissolve the horny layers of the epidermis. These compounds are able to separate, swell and macerate the keratinized epithelium, and in mycotic infections, slough off the outer infected areas of skin together with the spores and mycelium of the invading fungus. The most common examples of this class are salicylic acid and the alkalies, especially potassium and sodium hydroxide.

The most familiar agent of this group, salicylic acid, has found widespread use in Whitfield's Ointment which has the following formula:

Salicylic acid	6.00
Benzoic acid	12.00
Base	82.00

Another combination of salicylic acid is known as Phren's Foot Powder. This has the following formula:

Salicylic acid	5.00
Menthol	2.00
Camphor	8.00
Boric acid	50.00
Starch	35.00

Both of these combinations of salicylic acid are effective fungicides but they cause so much tissue degeneration that they must be considered as dangerous drugs. They have been used extensively, however, and unfortunately the largest use of these compounds has been more or less in the realm of self-medication. They may cause severe contact dermatitis especially in patients suffering from acute fungous infections.

The alkalies, such as sodium and potassium hydroxide, are efficient fungicides but they have enjoyed but limited use in the treatment of tinea pedis.

Antiseptic Agents

Due to the tremendous volume of material which has been written concerning medicinal agents that have antiseptic properties, it is almost impossible to discuss each compound separately. Since the emphasis of this study is upon the newer fungicidal agents, the discussion of antiseptic agents as fungicides, must, of necessity, be considerably limited and approached from a generalized standpoint.

The most popular members of this group are the following: 1:5,000 potassium permanganate, 1: 10,000 bichloride of mercury, phenol, 1: 500 aluminum acetate, 2 per cent gentian violet and other dyes, 1 per cent copper sulfate, tincture of iodine and the organic

mercurial tinctures such as mercurochrome, merthiolate and metaphen.

Collectively, these compounds are effective fungicides when used in the proper dilutions. They are especially efficient in eradicating the spores of the invading fungus.

A very popular liquid formula which was used extensively in the United States Navy during World War II is known as Castellani's Paint. This compound has the following formula:

Saturated alcoholic solution of basic fuchsin	10.00
5 per cent aqueous solution of phenol	100.00
Boric acid	1.00
Acetone	5.00
Resorcinol	10.00

This preparation is not very stable and may show signs of precipitation a short while after manufacture. It should be freshly prepared.

While these compounds are efficient fungicides, they may lead to dermatitis or blistering of the skin. This may be especially true of the alcoholic solutions such as tincture of iodine and the organic mercurial tinctures. These preparations become highly concentrated and may even show crystal formation due to the evaporation of alcohol.

Underwood (1946), in his classic study on over 400 patients suffering from overtreatment dermatitis, pointed out that the offender in 35 per cent of the patients was one of the organic mercurials, while phenol was responsible for 14 per cent of the cases. He went so far as to recommend that all mercurials should carry a warning statement on their label as to the possibility of sensitization of overtreatment dermatitis developing from their prolonged external use.

Sulfonamides

Both *in vitro* and clinical evaluation of the various sulfonamides as fungicidal agents have been disappointing. Some sulfonamide creams, notably sulfathiazole 5 per cent, have been of value in the treatment of secondary bacterial infections that may follow the fungous invasion. The sulfonamides have no other place in the therapy of fungous infections.

Antibiotics

The use of antibiotics in the treatment of fungous infections of the skin has been very limited and to some extent contraindicated. Schumann (1946) reported five cases of latent trichophyton infection which were activated by the use of penicillin. Hillegas (1945) found both penicillin and tyrothricin effective *in vitro*. Glio-toxin, aspergillic acid, clavacin, isoclavacin,

streptothricin, hemipyocyanin, allin and protoanemonin were all weakly fungistatic when clinically evaluated by Hopkins and associates (1946).

The complete inhibition of Trichophyton gypseum by dilutions of tyrothricin 1:5,000 was demonstrated by Stokes (1942). Gershenfeld demonstrated the fungistatic ability of tyrothricin by means of the spore germination technique (1947).

While this group of compounds has proved to be something of a panacea in the treatment of local and systemic infections due to bacteria, it does not appear that they will be of much value in the treatment of superficial mycotic infections.

Vaccinal Fungus Extracts

Trichophytin, which is essentially a vaccine obtained from species of Trichophyton, is available as both a diagnostic and therapeutic agent. It is generally prepared by trituration of a culture growth of Trichophyton with some solution, such as physiological salt solution, following which the mixture is heated for one hour at 60°C and a preservative is then added. While it is effective in demonstrating the heightened reactivity of the skin, it has not proved to be therapeutically effective in the desensitization of susceptible individuals.

Miscellaneous Agents

This represents a group of fungicidal agents that are difficult to present under other classifications. They have been included in this discussion due to their favorable mention in the literature.

Cresatin-Sulzberger

This is metacresylacetate, a colorless liquid which is soluble in oils but not in water. A well controlled clinical study proved this compound superior to Zephiran chloride (a quaternary ammonium compound), Whitfield's ointment(salicylic and benzoic acid),boric acid, and Iodolate, described below, in the treatment of superficial fungous infections of the feet (1946).

Iodolate

This is properly known as iodocholeate, an organic iodine compound in the form of a 20 per cent ointment. This compound was also evaluated in the clinical study mentioned above. Like other antiseptic agents, it is very apt to evoke chemical dermatitis and other untoward reactions of the skin.

Substituted Phenols

The addition of side chains to phenol has been found to increase its fungicidal activity. Of these compounds, two have been widely used and will be discussed separately.

Trichlorophenol

This has been found to be superior to many other fungicides but while many patients may tolerate it well, it more often irritates and aggravates the infected area. This was conclusively proved by Hopkins (1946). Since the chance of irritation is great when this compound is used, it is passing from favor in the treatment of superficial mycoses.

2,4, Di-nitro cyclohexyl phenol

This compound, which is widely used in agriculture as an insecticide, has gained some favor as a fungistatic agent. Twenty per cent solutions proved to be more fungistatic than other compounds,and Hopkins reported it highly fungistatic but also irritating (1946). Since it is a yellow dye and stains readily, its use as an external application is somewhat limited. It is of distinct value, however, in cases that do not respond to other treatment.

Quaternary Ammonium Compounds

Alkyl-dimethyl-benzyl-ammonium chloride (Zephiran - quaternary ammonium compound) and hexadecyl pyridinium chloride (Ceepryn - cationic detergent) are reputedly good fungistatic agents when tested in vitro. Dunn found Zephiran to be effective in dilutions of 1:25 against a series of fungi (1938). Clinical evaluation

of these compounds has revealed that they are inferior to other fungistatic agents especially Cresatin-Sulzberger and boric acid (1946).

THE FATTY ACIDS

It is interesting to note that while current interest in the fatty acids is focused mainly on their use as therapeutic agents, as far back as 1899 Clark reported on the effects of fatty acids on the germination of molds. In 1913 Kiesel demonstrated that the length of the carbon chain was important in determining fatty acid activity; he also proved that saturated fatty acids increased in activity as the number of carbon atoms up to eleven increased, and that the branched fatty acids were less active than those with straight chains and an equal number of carbon atoms. Hoffman, Schweitzer and Dalby (1939) investigated the fungistatic effect of fatty acids on bread molds and indicated that the effectiveness varied according to the length of the chain and concentration of the acid.

The use of the fatty acids in the treatment of superficial mycotic infections was introduced by Peck and his associates (1939). Their attention was directed to this type of treatment because their investigations convinced them that the human perspiration played a role as a protective mantle against infections in general and fungous infections in particular. They further postulated that while the fatty acids contained in human sweat might

not represent new and startling fungicidal agents, they would represent a more nearly physiologic approach to the therapy of fungous infections.

Analysis of a number of samples of human sweat revealed the following composition as indicated in Table 1.

Table 1

Constituents of Human Sweat (As reported by Peck, 1939)

Substance	Amount, per cent
Water	99.0200
Propionic acid	0.0062
Acetic acid	0.0096
Caprylic and/or caproic acid	0.0046
Sodium chloride	0.7000
Lactic acid	0.1000
Citric acid	0.0100
Ascorbic acid	0.0040
Urea	Trace
Uric acid	Trace

Since these workers encountered little difficulty in demonstrating the fungicidal power of whole samples of sweat, they determined to evaluate the fungicidal ability of the various constituents of sweat and also the sodium salts of the fatty acids contained therein.

Table 2 summarizes the results of this part of their study.

Table 2

Fungicidal Action of the Individual Constituents of Sweat on Tricophyton gypseum. (As reported by Peck, 1939)

Substance studied	Concentration Required to Prevent Growth
Lactic acid	0.20
Sodium lactate	3.00
Citric acid	0.30
Ascorbic acid	0.30
Acetic acid	0.05
Sodium acetate	5.00
Propionic acid	0.03
Sodium propionate	0.03
Caproic acid	0.009
Caprylic acid	0.03
Uric acid	Over 0.01 (Insol. in higher concentrations)
Urea	5.00

It should be pointed out that the above table indicates that with the exception of sodium propionate, the sodium salts of all of the acids studied were much less fungicidal than their corresponding acids. Peck pointed out that there is a possibility that some of

the volatile acids might be converted into salts on the cutaneous surface and in that event, the fungicidal action of the sweat would be greatly reduced.

A comparison of the figures in Tables 1 and 2 also indicates that lactic and caprylic acids are apparently present in sufficient concentrations to be of practical value as fungicides.

Peck and Rosenfeld (1938) studied a series of fatty acids beginning with formic acid with 1 carbon atom and going up to capric acid with 10 carbon atoms. The unsaturated fatty acid, undecylenic, was also included in this survey. Several isomers of the fatty acids were also evaluated. The following table indicates the results obtained:

Table 3

Effects of fatty acids, their salts and isomers, on the growth of Tricophyton gypseum. (Peck and Rosenfeld, 1938)

Substance Studied	Chemical Formula for Acid	Concentration Required to Prevent Growth
Formic acid	CHO_2	0.03
Sodium formate		0.5
Acetic acid	$C_2H_4O_2$	0.03
Sodium acetate		5.00
Propionic acid	$C_3H_6O_2$	0.03
Sodium propionate		0.03
Butyric acid	$C_4H_8O_2$	0.01
Sodium butyrate		0.05
Iso-butyric acid	$C_4H_8O_2$	0.07
Valeric acid	$C_5H_{10}O_2$	0.003
Sodium valerate		0.5
Iso-valeric acid	$C_5H_{10}O_2$	0.03
Methylethyl-acetic acid	$C_5H_{10}O_2$	0.03
Caproic acid	$C_6H_{12}O_2$	0.009
Caprylic acid	$C_8H_{16}O_2$	0.03
Pelargonic acid	$C_9H_{18}O_2$	0.009
Capric acid	$C_{10}H_{20}O_2$	0.009
Undecylenic acid	$C_{11}H_{20}O_2$	0.005
Sodium undecylenate		0.0009

It can be seen from Table 3 that in every instance the isomers of the fatty acids studied were much less fungicidal than the normal acids,and the normal salts were also less fungicidal than their corresponding acids. Sodium propionate, however, was as effective as propionic acid as a fungicide. The importance of double bonds in the chain was demonstrated by the activity of undecylenic acid and its sodium salt. Sodium undecylenate proved to be the most powerful fungicide of the series reported in Table 3. With the exception of pelargonic acid, the fatty acids with an odd number of carbon atoms tended to be more fungicidal than those with an even number.

Perhaps the solid groundwork for therapy resulting from the investigations of Peck and associates would have created little interest had it not been for World War II. The war and marching soldiers gave fresh significance to the prevention and treatment of infections of the feet, especially "athlete's foot." A tremendous program of clinical investigation of the fatty acids, stimulated by the United States Army and Navy, resulted in many new compounds being made available for the treatment of superficial mycotic infections. These will be discussed and evaluated in detail.

CLINICAL EVALUATION OF THE FATTY ACIDS

While *in vitro* methods of testing may be worthwhile in the preliminary evaluation of various compounds, it is the clinical study alone that reveals the true therapeutic efficacy of medicinal agents.

In their original report, Peck and his associates presented evidence on the use of a limited number of fatty acids and salts in the treatment of superficial fungous infections (1939). These workers evaluated 10 per cent sodium propionate in 50 per cent alcohol, 15 per cent sodium propionate in venetian talcum and 15 per cent sodium propionate in anhydrous wool fat and petrolatum. Other mixtures of fatty acids were also included in this study. Their results were encouraging and stimulated other workers to investigate, on a more extensive scale, other fatty acids and their salts. These studies will be described.

Propionic Acid and Sodium Propionate

An investigation of propionic acid and sodium propionate was undertaken at the Johns Hopkins University at the direction of the Office of Scientific Research and Development of the United States Government. This clinical investigation was stimulated by the reports of Peck and his co-workers and also by reports that sodium

and calcium propionate - trade name "Mycoban" exerted a retarding effect on molds in dairy products.

In this study a 10 per cent sodium propionate ointment, powder and solution were investigated. A total of 55 midshipmen from the United States Naval Academy, all of whom suffered from "athlete's foot", were treated and observed at regular intervals for a period of twenty weeks. This study indicated that sodium propionate was an effective fungicidal agent and was also non-irritating to the skin.

Later, these same workers investigated mixtures of propionic acid and sodium propionate in ointment form and found the mixture to be superior to plain propionic acid or mixtures of undecylenic acid and zinc undecylenate (1945).

Undecylenic Acid and Its Salts

One of the most extensive clinical investigations relative to the evaluation of fatty acids as fungicidal agents, was carried out by Hopkins and his co-workers (1946) at Fort Benning, Georgia. This study included as subjects, a large number of soldiers who were in active training under conditions that were most appropriate for the growth and spread of the organism causing "athlete's foot". Screening tests were made with over 70 different fungicides and most of these

agents were tested in different concentrations and in various vehicles. Altogether, a total of over 400 preparations were evaluated. Among the various compounds studied, the following are of importance because of their widespread use: undecylenic acid, propionic acid, salicylic acid, benzoic acid, tri-chlorophenol, sodium caprylate, zephiran, iodine and tincture of merthiolate.

This investigation revealed that 5 per cent undecylenic acid was the most effective of the various agents evaluated. This conclusion was made due to the high percentage of effectiveness and the low incidence of irritation possessed by this unsaturated fatty acid. Other workers, notably Sulzberger, Shaw and Kanof, have indicated the value of a combination of 5 per cent undecylenic acid and 18 per cent zinc undecylenate (1945). The zinc salt is purported to break down slowly and to liberate undecylenic acid, thus making the combination effective for a longer period of time than the preparations embodying undecylenic acid alone.

These investigators (Sulzberger, Shaw and Kanof) employed a unique and scientific method for evaluating the various agents that they studied. Based on the knowledge that tinea pedis is generally a bi-lateral infection, they instructed the subjects to apply one type of medication to the right foot and a different

type to the left foot.

Among the compounds studied were combinations of 5 per cent undecylenic acid and 20 per cent zinc undecylenate in both ointment and powder forms and 20 per cent sodium propionate in both ointment and powder forms.

The results of this study indicated the superiority of the undecylenic acid-undecylenate combination over the other agents studied and particularly, sodium propionate. It should be pointed out, however, that the plain 5 per cent undecylenic acid preparation reported on by Hopkins (1946) was not included in this clinical program.

Another interesting conclusion resulting from this research was that the prophylactic use of the undecylenic acid-zinc undecylenate powder was able to reduce the incidence of dermatophytosis by 85 per cent.

Caprylic Acid and Sodium Caprylate

Due to a scarcity of cocoanut oil which is the source of caprylic acid, there has been a lack of extensive *in vitro* and clinical evaluation of this fatty acid and its salts.

One outstanding piece of clinical research was carried out at the United States Naval Academy by Keeney and his associates (1945). They investigated the efficacy of a 10 per cent sodium caprylate ointment on a series

of midshipmen all of whom showed evidence of tinea pedis. Forty-six of these men were treated with the sodium caprylate ointment and a control group of forty-five were treated with the ointment base alone (the base was composed of "Carbowax", n-propyl alcohol and diethylene glycol mono-ethyl ether).

These workers concluded that the clinical results obtained with 10 per cent sodium caprylate ointment were significantly superior to the results that they had previously obtained and reported on relative to the use of propionic acid or its salts or undecylenic acid or its salts. This conclusion is of interest because the latter study did not include the propionic or undecylenic acid compounds or combinations.

Combinations of Fatty Acids

Lately, Peck and Russ (1947) have reported on mixtures of sodium propionate, propionic acid and sodium caprylate. These workers theorized that since both propionic and caprylic acids are found in the sweat, the combination of the two acids should be more effective than either one of them separately. A clinical study of the mixture substantiated this hypothesis.

Based on the available data relative to the clinical evaluations which have been made, certain conclusions can be drawn regarding the fungicidal agents

that have been discussed. They are as follows:

1. The fatty acids, especially propionic, caprylic and the unsaturated undecylenic, are more effective than agents which have previously been used in the treatment of Tinea pedis, such as Whitfield's ointment, Castellani's Paint and the mercurial tinctures.
2. The fatty acids are also safer in that they do not cause sensitization of the skin nor evoke other untoward reactions such as chemical dermatoses.
3. Since many of the fatty acids are found in the sweat, they represent a physiological approach in the treatment of superficial dermatophytosis.
4. Clinical studies have not been extensive enough to demonstrate the striking superiority of any single fatty acid or combination of acids or salts thereof.

AGENTS USED IN THE TREATMENT OF TINEA CAPITIS

The widespread epidemic of tinea capitis that occurred in Hagerstown, Maryland in 1944 and 1945, offered an unusual opportunity to evaluate the therapeutic agents that were advocated for this condition. Since all but approximately 1 per cent of the cases treated were caused by *Microsporum audouini*, the most resistant type of infection, the compounds evaluated were given a severe test.

During the epidemic, Schwartz and his co-workers of the United States Public Health Service tried a number of compounds including copper oleate, colloidal iodine, pentachlorphenol, salicylanilide and copper undecylenate (1946). They reported that the two most efficacious compounds were salicylanilide and copper undecylenate. These are discussed below.

Salicylanilide

Salicylanilide was used in the form of a 5 per cent ointment. During the epidemic, 83 children were treated with this compound and 47 of these patients were reportedly cured.

Copper Undecylenate

The copper compound was chosen due to the known fungicidal activity of copper and the failure of zinc

undecylenate to cure this condition in previous tests. A saturated solution of this salt (approximately 10 per cent) in an ointment base was found effective in 24 of the 42 cases on which it was used.

Other Compounds

Co-indicental with the extensive study made at Hagerstown, Maryland, other workers, notably Strickler, Carrick, Lewis and Rothman have made contributions which are worth discussing. Due to the variations in the reports of these workers, the agents that they evaluated will have to be discussed together. While some separation can be made, considerable overlapping is unavoidable.

Endocrine Therapy

Encouraged by the reports of Poth, Kaliski and Barman (1942) that indicated good clinical results with the use of estrogenic substances, Lewis and his associates (1946) were persuaded to evaluate the local application of estrogens and androgens to the infected scalps.

They tested the possible effect of testosterone, diethyl stilbesterol, ethynil estradiol, desoxycorticosterone and alpha estradiol on Microsporum audouini in vitro. They found that none of these hormones were markedly fungicidal or fungistatic against

this particular organism.

Clinically, they evaluated the following estrogens and androgens on some 23 patients:

1. Diethylstilbesterol - 1 mg. in petrolatum
2. Testosterone - 3 mg. in an ointment base
3. Estradiol - 1 mg. per day applied topically.

These preparations were rubbed in once daily.

The workers concluded that the results of estrogen therapy were disappointing and that roentgen ray epilation of the scalp was a superior treatment.

Strickler's Solution

Strickler has suggested a unique mixture which he advocates for the treatment of this condition (1946). The ingredients of his solution and the rationale behind the use of the various components are as follows:

Solution		Rationale
Ingredient	Per Cent	
Iodine	78.0	fungistatic and possibly fungicidal
Red blood cells	2.0	essential for "photo-synthesis"
Sodium chloride	1.9	isotonic diluent
Chlorophyll	2.0	catalyst and photo-sensitizer
Decresol	0.07	wetting agent
Magnesium dioxide	10.0	source of oxygen
Spleen extract	0.005	an enzyme capable of transferring peroxide oxygen to oxidizable products.

Strickler assumed that the iodine molecule became activated when exposed to a quartz mercury vapor arc and that most of the energy is utilized to photosensitize the iodine molecule. This iodine molecule so treated, has a more intensive effect.

A clinical evaluation of this mixture, on a series of 115 patients resulted in a cure in 74 of the subjects treated.

It should be pointed out that Strickler studied only his own compound and did not investigate the value of other agents during his study.

Copper Oleate

Carrick (1946) recognized the need for some method of local treatment of tinea capitis that would not irritate or sensitize the scalp. His first approach to the problem was the use of copper oleate. He clinically investigated a 15 per cent ointment of this compound and found it effective in 53 per cent of the cases on which it was tried.

Fatty Acids

Carrick also studied mixtures of the propionates and undecylenates to a limited extent (1946).

A mixture of sodium propionate, 16.4 per cent,

propionic acid, 3.6 per cent and zinc propionate, 5 per cent, proved effective in 40.6 per cent of the cases on which it was tried.

A preparation containing undecylenic acid, 5 per cent and zinc undecylenate, 20 per cent, proved efficacious in 40 per cent of the tinea capitis patients. Carrick concluded that the fatty acid preparations might be employed in certain cases providing that other factors were observed. He indicated that manual or roentgen ray epilation of the infected hairs was necessary along with the use of the therapeutic agent at least twice daily.

Summary

While tinea capitis has not received the widespread attention that has been given to the therapy of other superficial fungous infections, certain progress has been made in the treatment of this condition.

Once again, the fatty acids and their salts have proved to be safe and effective therapeutic agents. It is even possible that these same fatty acids that are effective in the treatment of "athlete's foot" will prove to be the physiological approach to the treatment of infections of the scalp. Rothman and his associates have been able to isolate from "hair fat", highly active normal aliphatic monobasic acids including pelargonic and tridecanoic. It is possible that these acids, as well

as others of the same series, are responsible for the spontaneous cure of some cases of tinea capitis at the onset of puberty. It is at that time that the sebaceous glands of the scalp start to secrete a sebum which contains higher concentrations of low boiling, saturated fatty acids with selective fungistatic and fungicidal action.

It would seem that the most efficient of the agents that have been reported on in the literature are salicylanilide and copper undecylenate. Based on the extensive clinical work of Schwartz and his group (1946) these two agents must be accorded prime consideration in the therapy of tinea capitis.

THE MECHANISM OF ACTION OF THE AGENTS DISCUSSED

This topic is one of tremendous scope and a complete discussion of the modes of action of fungistatic or fungicidal agents is beyond the intent of the writer. Yet any discussion of agents of this nature would lack completeness if some explanation were not included.

The ensuing discussion is not, then, a complete dissertation on the subject. Rather, it is an attempt to classify the various agents into categories that are well recognized.

Much of the material to be presented is based on the known activity of these agents on microorganisms.

The classification and a short discussion of each group follows herewith:

Antibiotics

The exact mechanism of antibiotic activity is not at the moment clearly defined. These agents, products of living microorganisms, may work by setting up various types of antagonism that can exist in the microscopic world. Two possibilities are that they interfere with the enzyme systems of the living mold, or that they interfere with the utilization of certain growth stimulating substances. Either of these factors might

well be the answer to the question of antibiotic activity.

The antibiotics discussed in this thesis were penicillin and tyrothricin.

Astringents

These agents cause a local shrinking of tissue, a diminution of exudates and a blanching of the skin. They act as protein precipitants but since these compounds do not penetrate well, their activity is relatively weak and is confined to the surface of the cells. When astringents act, the affected tissue cells lose some of their permeability but they are not killed.

This group is mentioned here due to the extensive use of a solution of aluminum acetate (Burow's solution) in the therapy of certain superficial mycotic infections. These agents probably have only an indirect effect on the fungi, in that they change the environment on which the organism is endeavoring to grow.

The only compound of this type that has been discussed is solution of aluminum acetate.

Cationic Detergents - Surface Active Agents

These compounds decrease the interfacial tension that exists between the mold cells and their environment. When cationic agents are adsorbed on the surface of the microorganism, they seem to exert an inhibitory effect on the metabolism and viability of the organism. It is

postulated that the combination of the cell and adsorbed cationic agent prevents the normal absorption of specific nutritional substances.

Agents of this sort which were discussed in the study were cetyl pyridinium chloride (Ceepryn) and benzalkonium chloride (Zephiran).

Chemo-Therapeutic Agents

The action of sulfonamides on fungi has not been confirmed, although Lewis and Hopper (1941) reported sulfanilamide to be effective in vitro against several species of Trichophyton. Their observations have not been substantiated by clinical evaluations.

Any fungistatic or fungicidal action that these compounds might possess would presumably be due to the same effect that they have on bacteria. The sulfonamides exert their effect on bacteria by interfering with the utilization of p-aminobenzoic acid and essential cell metabolite.

This group is included in the discussion because mention was previously made of the sulfonamides as agents that are now used in some part of the treatment of "athlete's foot."

The Fatty Acids

It is generally accepted that the antiseptic properties of most acids are due chiefly to the hydrogen

ion concentration they produce.

In respect to the fatty acids, on the other hand, it has been demonstrated that the normal acids are more fungicidal than their salts or homologues and that the acids with even numbered carbon atoms are less fungicidal than those with odd numbers of carbon atoms in their chain.

To date, there is nothing definite in the literature relative to the exact mechanism of action of these compounds. Cowles (1941) has attributed their action simply to the hydrogen ion concentration that they produce. His theory has not been substantiated at this time.

This thesis includes a discussion of the following fatty acids and their salts: caprylic, caproic, propionic and undecylenic.

Iodine

According to Frobisher (1944), iodine acts by combining with the constituents of protoplasm resulting in a combination that does not have protoplasmic properties.

Frobisher also suggests that iodine is able to liberate "nascent" oxygen from water which would also contribute to its fungicidal effect in an indirect manner.

Tincture of iodine, iodocholeate and an activated iodine solution (Strickler's solution) were discussed in this thesis.

Metals and Their Salts

The fungicidal activity of the heavy metals and their salts is due to the affinity of the cations for protein material.

According to Jordan and Burrows (1946), the bivalent cations are more toxic than the monovalent cations and the salts of the heavier metals are more toxic than those of the lighter metals.

All of these compounds are efficient when they are able to precipitate the constituent proteins of the cells.

Compounds of this type that have been discussed are bichloride of mercury and copper sulfate.

Organic Dyes

Many organic dyes are able to exert a deleterious effect on the growth of microorganisms. They are said to prevent the multiplication of the cells so that their effect is fungistatic rather than fungicidal in nature.

This class includes the organic mercurial tinctures, where there is also heavy metal activity, and certain dyes such as gentian violet and fuschin. These have been evaluated in this thesis.

Oxidizing Agents

Many chemical compounds, such as potassium permanganate and hydrogen peroxide, owe their fungicidal ability to the releasing of "nascent" oxygen which has a

deleterious effect on the growth of the organisms.

The above mentioned compounds were discussed earlier in the thesis.

Phenols and Cresols

There are two schools of thought as to the mode of action of phenol and phenol-like compounds.

One states that these compounds act on the proteins of the cell with the formation of insoluble proteinates and a subsequent precipitation of the proteins of the protoplasm. The other believes that the action is more physical than chemical, the phenolic compound having the ability to dissolve in coagulated proteins. The fungicidal action is thought to be due to the ability of the compound to penetrate the cells in the form of a colloidal solution. After penetrating the cell, the compound presumably precipitates the cell protoplasm and thus exerts its fungicidal effect.

Compounds of this type that were previously discussed are Cresatin, trichlorophenol and 2,4, Dinitrocyclohexyl phenol.

Other Actions

The popular combination of salicylic acid and benzoic acid (Whitfield's Ointment) probably exerts a dual effect.

The salicylic acid content exerts a keratolytic

effect and causes the sloughing off of tissue containing mold spores and mycelia.

The benzoic acid in the formula apparently exerts an antiseptic effect by the activity of the whole molecule and is not due to any dissociation into cations.

SECTION B

ORIGINAL INVESTIGATION

OBJECTIVES OF THE INVESTIGATION

Foreword

The original investigation was inspired by the reports of new and improved therapeutic agents for the treatment of superficial fungous infections. A subsequent survey of the literature revealed a multiplicity of methods used for the *in vitro* evaluation of fungicidal agents. None of these methods seemed to be entirely without fault. Further, while the newer fungicidal agents had been extensively evaluated by these methods *in vitro* and also in "the crucible of the clinic", little attention had been paid to the selection of the most suitable vehicle in which to incorporate these compounds.

The original investigation was based on a desire to develop a simplified method for the *in vitro* evaluation of fungicides and to determine the relative value of various ointment bases as possible vehicles for the improved therapeutic agents. Like all research, this investigation led to many other channels of exploration, avenues that have not all been investigated at this time. Perhaps true research should not lead to easy conclusions. It is the opinion of this investigator that the ensuing report represents a foundation upon which future studies can be based.

The various objectives of the investigation may be summed up as follows:

1. To develop, if possible, a more practicable method for the *in vitro* evaluation of fungistatic and fungicidal agents.
2. To determine the sporulation time of a culture of *Trichophyton mentagrophytes* 9129.
3. To compare the relative value of various ointment bases as vehicles for fungicidal agents.
4. To compare the effect of the addition of the medication at various intervals after the plates have been seeded.
5. To determine the duration of fungicidal activity of various agents.
6. To determine the effect of pH of the preparation on its fungicidal activity.

A discussion of the various objectives of the investigation follows.

I - A PROPOSED METHOD FOR THE IN VITRO EVALUATION OF FUNGISTATIC AND FUNGICIDAL AGENTS

A literature survey indicated that the methods which have been used, and are still being used in many cases, for the evaluation of fungicidal agents, fall into the following categories:

1. Concentration Methods
    - A. Pellicle Method (broth concentration).
    - B. Agar Concentration.
2. Modified F.D.A. Methods
    - A. Phenol Coefficient Method.
    - B. Modified Agar Plate Methods.
    - C. The Burlingame and Reddish Method.

In the concentration methods, varying amounts of the agents being tested are added to Sabouraud's broth or agar and the amount necessary to inhibit growth is determined. While these methods give a fairly accurate measure of the amount of the agent necessary to prevent the growth of the organism, they are not too practical and they do not simulate conditions encountered in vivo.

Modifications of the phenol coefficient technique have been used to evaluate fungicidal agents. All of these modifications are based on the use of a series of dilutions of phenol and the fungicide to be tested. The

respective dilutions are then inoculated with a suspension of a Trichophyton species. Transfers are made from the various dilutions to culture media to test for viability of the culture.

These determinations are not accurate and the phenol coefficient test, or its various modifications, does not seem suitable for the evaluation of fungicides.

Of the various Agar Plate modifications, the method proposed by Burlingame and Reddish seems most practicable and scientific. Their suggested technique involves the use of five test organisms: Epidermophyton interdigitale, Trichophyton purpureum, Trichophyton gypseum, Epidermophyton inguinale and Trichophyton rosaceum(1939).

Each of the organisms is streaked over the entire surface of Sabouraud's agar. The plates are then incubated at room temperature for five days. The cultures are then cut into 1 cm. squares, or into disks(by means of a cork borer) 1 cm. in diameter. The fungicide to be tested is poured over the surface of the cultures so as to flood the plates entirely, which requires 15 cc. or more for each plate. After 5, 15, and 30 minutes, one of the squares or disks of culture and agar from each plate is removed and placed in 10 cc. of sterile broth. The excess fungicide is washed out of the matted culture by

shaking the broth tube lightly for 5 minutes. At the end of this time the block of culture is removed from the broth and spread with the culture side down over the surface of a sterile slant of Sabouraud's agar. These slants are then incubated at room temperature for 3 weeks and observed for growth.

While this method may represent the ideal manner in which to evaluate fungicidal agents, it is too time consuming and cannot be adapted for semi-solid preparations such as ointments.

Shapiro and Rothman (1945) have pointed out that a good fungicidal agent should be able to kill fungi and also penetrate to the location of the fungous infection. Accepting their tenets, a good method should test for penetration as well as for the killing power of the agent. The test for penetration is especially important with the decided trend toward the use of water-miscible bases as vehicles for medicinal agents.

Since none of the methods previously used were suitable for the investigation, it seemed desirable to develop a new method using the agar plate technique as a starting point.

The following technique was developed and has been employed for the <u>in vitro</u> evaluations reported herein.

Description of Method

A. Cultures of T. mentagrophytes are grown in Honey Broth U.S.P. XII. The formula for this medium is as follows:

Peptone	10 Gm.
Honey	60 cc.
Distilled Water, a sufficient quantity	
To make	1000 cc.

A suspension of a culture of this sort which is at least four days old, is uniformly suspended by "aspiration" with a hypodermic syringe.

B. This suspension is then dropped on the surface of Sabouraud's maltose agar in a uniform pattern. This is accomplished by utilizing a 1 cc. Tuberculin syringe with a 1½ inch 22 gauge needle. The pattern used is shown in Figure 4, page 53.

The plates are placed over the pattern and the suspended culture dropped over the red dots of the pattern. These dots are 1 cm. apart. A resultant growth is shown in Plate 1, page 54.

C. Twenty-four hours after the "seeding" of the plates, 1 cc. of the agent to be tested is added directly to the center of the plate by means of a hypodermic syringe.

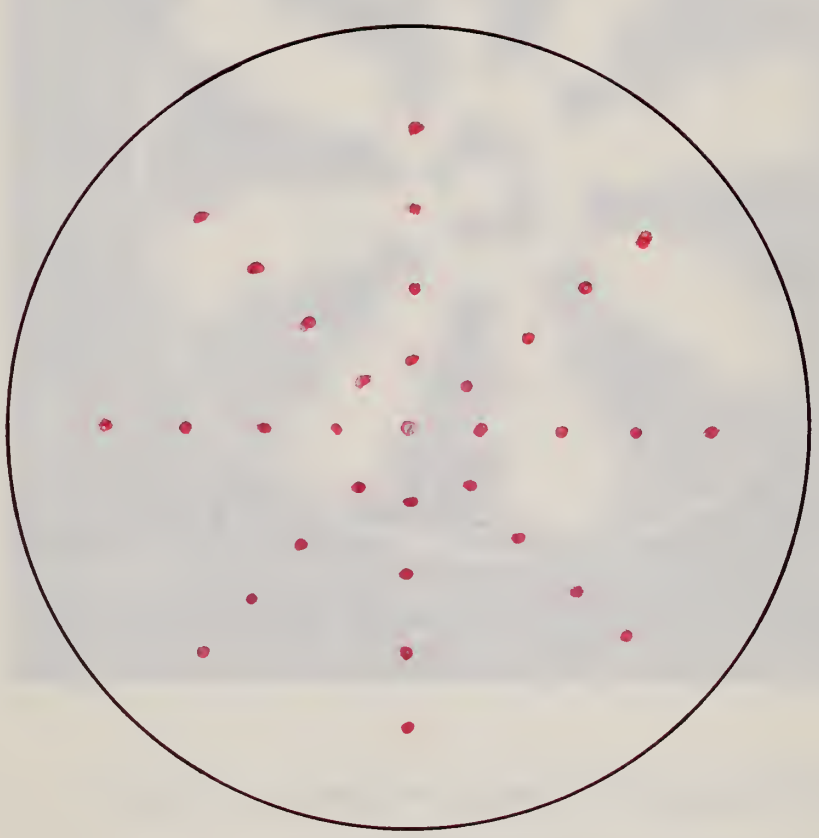

Fig. 4 - Pattern used in seeding the plates.

Plate 1 - Uniform growth of Trichophyton mentagrophytes resulting from seeding of plates as shown in Fig. 4. Photograph taken 30 days after plate was seeded.

D. The inhibition produced by the medication is measured each day for at least one week. Measurements are made in millimeters and are compared with control plates containing only the base of the combination being tested.

E. Temperature of the above test - 20°C.

Conclusions

Having had opportunity to work with this method for the past year, some conclusions can be made at this time.

There are certain attributes in favor of the use of this method. These are:

1. It does not involve a difficult technique.
2. It is not time consuming.
3. It is superior to the concentration methods, in that the medication can be evaluated while it is incorporated in various bases.
4. It is a uniform method due to the measured amounts of inoculum that are seeded at standard intervals on the plate and the measured amount of medication added.

In common with other methods of testing, this method has several shortcomings. They are as follows:

1. This method does not adequately measure penetration, which is an important factor in the treatment of superficial fungous infections. This

same criticism can be levelled at any *in vitro* method that utilizes a non-living medium such as agar. It is possible that this objection can be overcome.

2. Since this study has not been correlated with any clinical study, the results obtained by the use of the method are hardly indicative of what might occur on *in vivo* evaluation of the same compounds. Keeney (1943) has stated "It is impossible to predict or anticipate the clinical effectiveness of fungistatic or fungicidal agents from the results of *in vitro* experiments".

II - DETERMINATION OF THE SPORULATION TIME OF A CULTURE OF TRICHOPHYTON MENTAGROPHYTES 9129

In order to standardize certain steps of the method used for *in vitro* testing, it was first proposed to determine the sporulation time for the culture used in the evaluations.

Method

A culture of *Trichophyton mentagrophytes* 9129 which was obtained from the American Type Culture Committee was grown on Sabouraud's maltose agar and in Honey Broth, U.S.P. XII, at 20°C. A photograph of this culture is shown on page 58.

Both of these cultures were studied at twenty-four hour intervals. Studies were made with a dissecting microscope and also by transferring some of the growth to a glycerin-alcohol mount for high-dry observation. It was found in both instances, that a few spores formed at the end of forty-eight hours but that copious amounts of spores were not formed until seventy-two hours had elapsed.

These results were substantiated by a further study using a modified Henrici culture. This culture was prepared as follows:

Melted Sabouraud's maltose agar was allowed to

Plate 2 - Culture of Trichophyton mentagrophytes 9129 growing on Sabouraud's maltose agar slant

harden in the bottom of a deep-well slide. This was then inoculated with a transfer of the mold and high-dry observations were made every twenty-four hours. Abundant mycelia were found at the end of twenty-four hours, a few spores at forty-eight hours and numerous spores were present at the seventy-two hour observation.

III - EVALUATION OF VARIOUS OINTMENT BASES

It is unfortunate that this part of the discussion cannot be prefaced with the classical article on "Vehicles In Topical Dermatologic Therapy" by C. Guy Lane and I. H. Blank (1946). Briefly, these two investigators point out that too little consideration is often given to the vehicle and the preparation of dermatologic therapeutic material for local use.

It has been shown repeatedly that when Hydrophilic Petrolatum, U.S.P. XIII, is used as a base for certain therapeutic compounds, such as Whitfield's Ointment, the preparation is just as effective in lower strength as the full-strength preparation in a petrolatum base.

Moreover, the so-called "washable ointment bases" have many attributes that pre-dispose to their use. They can be easily removed from the skin with water alone; they may easily be removed from bed linen and clothing and they possess a superior cosmetic effect.

In this investigation six different bases were evaluated. The composition of these bases is shown on pages 61 and 62.

Procedure

Five per cent undecylenic acid was added to each of the following bases:

Base No. 1. M.C.P. Base

Glyceryl Monostearate	8.00
Spermaceti	7.00
Theobroma Oil	5.00
Liquid Petrolatum	8.00
Distilled Water	60.00
Glycerin	5.00
Preservative	0.10

Base No. 2. "Beeler Base"

Cetyl Alcohol	15.00
White Wax	1.00
Glycerin	10.00
Sodium Lauryl Sulfate	2.00
Distilled Water	72.00

Base No. 3. Hydrophilic Ointment U.S.P.XIII

Stearyl Alcohol	250.00
White Petrolatum	250.00
Glycerin	120.00
Sodium Lauryl Sulfate	10.00
Methyl Parahydroxybenzoate	0.25
Propyl Parahydroxybenzoate	0.15
Distilled Water	369.00

Base No. 4. Typical Vanishing Cream Base

White Wax	20.00
Mineral Oil, Heavy	122.00
Triethanolamine	4.00
Aerosol O.T.	0.10
Water	200.00

Base No. 5. Hydrophilic Petrolatum U.S.P.XIII

Stearyl Alcohol	30.00
Cholesterol	10.00
White Wax	80.00
Wool Fat	150.00
White Petrolatum	750.00

Base No. 6. White Petrolatum Base U.S.P.XIII

Wool Fat	5.00
White Wax	5.00
Petrolatum	90.00

All of the various bases were evaluated by the method described in the first part of this investigation. The organism that the agents were evaluated against was T. mentagrophytes. In the various determinations that were made, each of the bases was used as a control.

The results of these tests indicated a clear-cut superiority of the "washable-ointment" bases over the petrolatum types.

Typical results are shown in Table 4, page 64.

It can be readily seen from studying the table, that of these "washable" bases, the so-called "Beeler Base" proved to be the best vehicle not only for the carrying of the medication, but for the liberation of the medication when needed. The agent in "washable bases", numbers 1, 2, 3 and 4 in Table 4 exhibited marked inhibitory activity. The results obtained with the greasy-type bases were as follows: Number 5 in Table 4 did not manifest much inhibition and

number 6 in Table 4 failed to inhibit at all. The greasy-type bases probably mask the action of the medicinal agents and do not liberate the medication into the surrounding areas.

These results are in agreement with the findings of Gershenfeld and Brillhart(1939) who reported that bactericidal agents in water-miscible ointment bases produced greater inhibition zones than older types of bases when tested by Agar Plate technique against Staphylococcus aureus.

Plates 3, 4 and 5, pages 65, 66 and 67 show some of the results obtained during the aforementioned study.

Table 4

Comparative Effectiveness Of Undecylenic Acid In Various Ointment Bases

Base	Inhibition in Millimeters At 96 Hours
1	10
*Control	none
2	20
*Control	none
3	18
*Control	none
4	6
*Control	none
5	5
*Control	none
6	none
*Control	none

*Controls represent plain base without undecylenic acid.

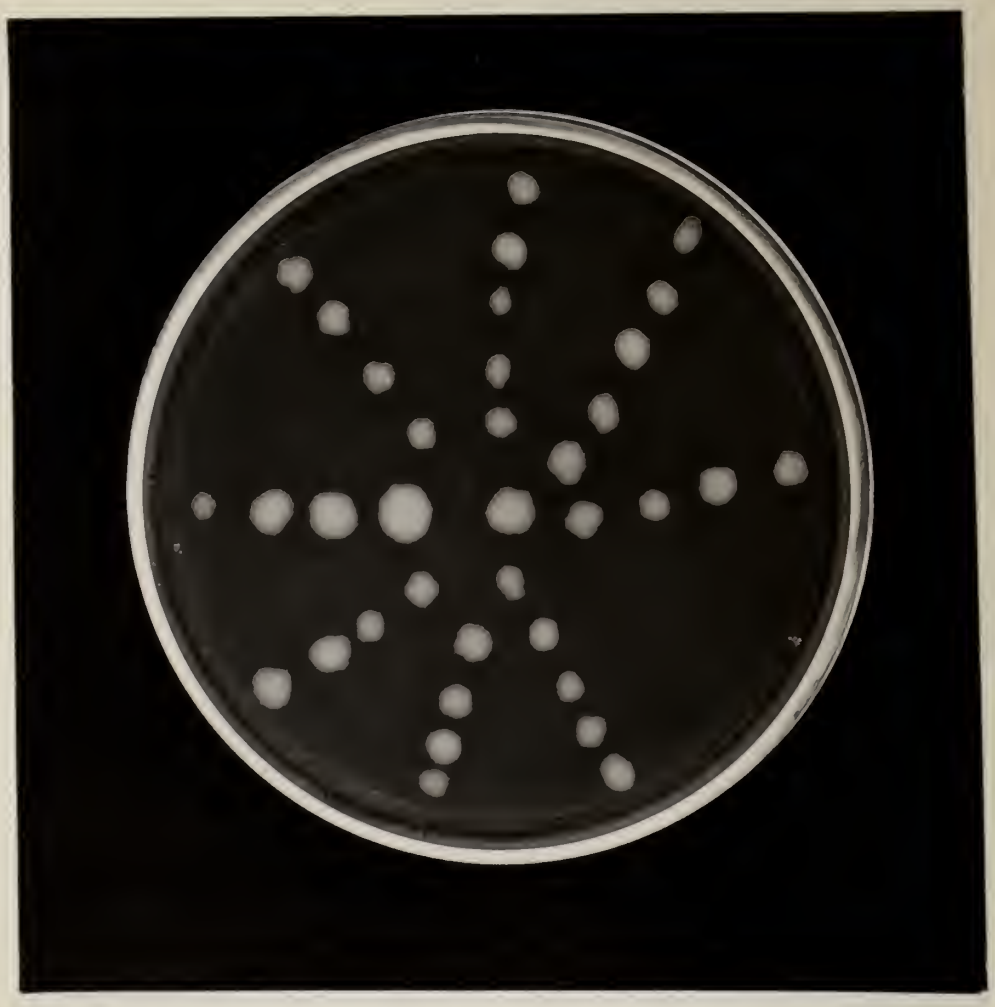

Plate 3 - Control plate showing growth resulting from the seeding of a plate with culture of T. mentagrophytes. Photograph taken 5 days after plate was seeded. Compare with Plate 5.

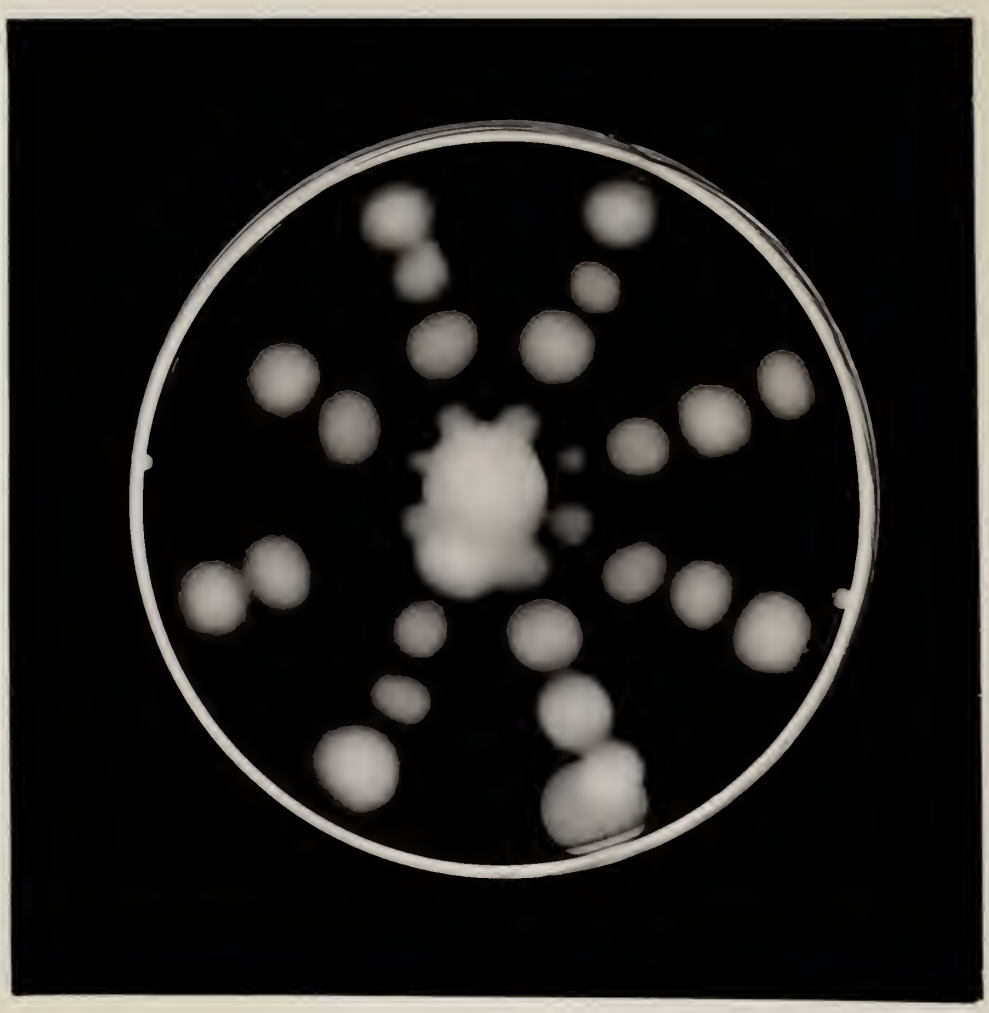

Plate 4 - The result of the addition of a water-miscible base without the addition of medication to a culture of T. mentagrophytes. Note the colonies surrounding the base in the center of the plate. Photograph taken 8 days after plate was seeded.

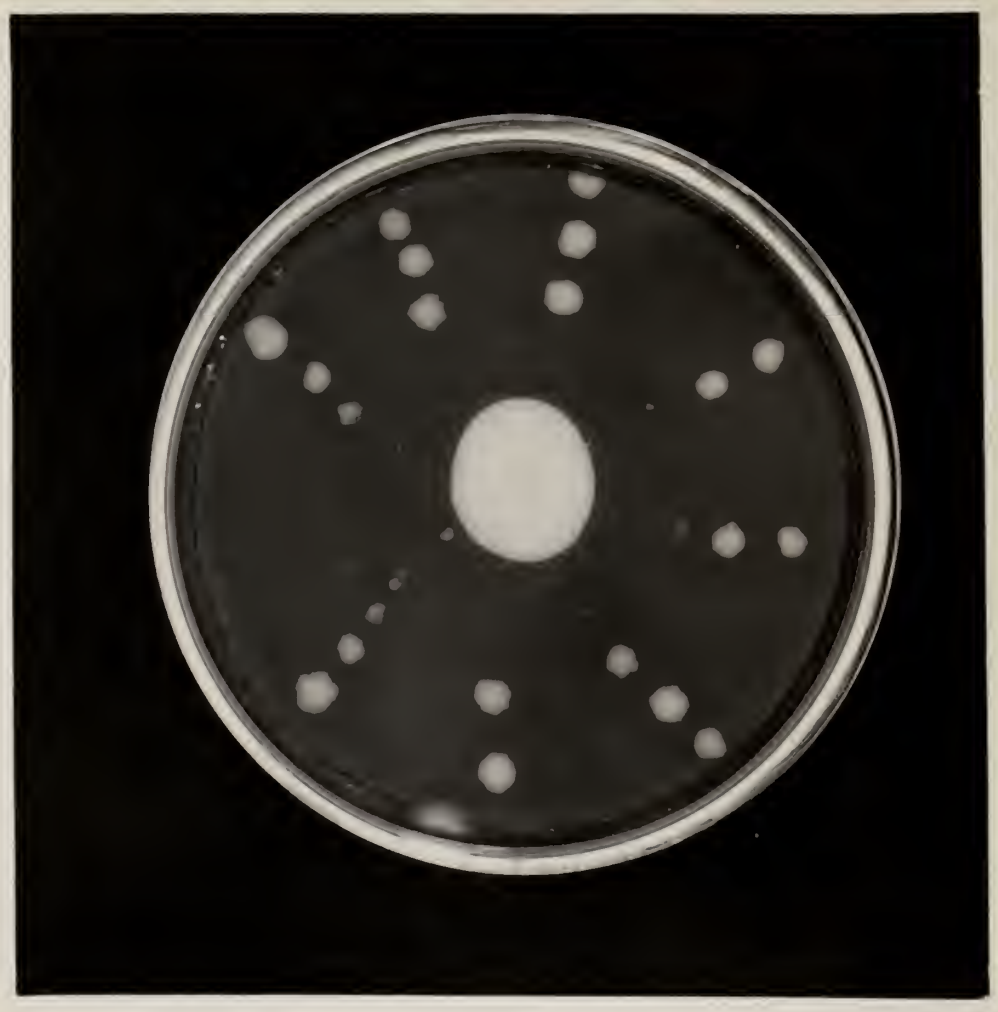

Plate 5 - The result of the addition of 5 per cent undecylenic acid in a water-miscible base to a culture of T. mentagrophytes. Note wide zone of inhibition. Photograph taken 5 days after plate was seeded. Compare with Plate 3.

IV - THE EFFECT OF THE ADDITION OF THE MEDICATION AT VARIOUS INTERVALS AFTER THE SEEDING OF THE PLATES

One of the factors that undoubtedly modifies the in vivo activity of fungistatic or fungicidal agents, is the relationship between the time of infection and the time that the medication is added.

This factor is also one that modifies the results of the in vitro evaluation of such agents. It is possible in the laboratory, however, to control this variable within certain limitations. Despite this fact, the time relationship between the inoculation of the medium and the addition of the medication is too often neglected in most in vitro methods of testing. Indeed, it is the custom in most of these tests, to add the medicinal agent at the same time that the inoculum is added. It seems hardly possible that the organism has opportunity to get a "foothold" before it is exposed to the adverse conditions offered by the medicament.

It was with these factors in mind, that this investigation was directed to a determination of the correct time to add the medication after the inoculation of the plates.

Method

A series of Sabouraud's maltose agar plates were

inoculated with a suspension of the test organism - T. mentagrophytes.

An ointment base containing 5 per cent undecylenic acid was added to these plates at the following time intervals:

A. Added at the same time as inoculation.

B. Added 24 hours after inoculation.

C. Added 48 hours after inoculation.

D. Added 72 hours after inoculation.

All of the plates were incubated at 20°C from the time of inoculation until the conclusion of the test. Plates were examined at twenty-four hour intervals. Zones of inhibition were measured in millimeters.

Results

A. No growth on the plate.

B. Wide zone of inhibition - 18 millimeters, uniform colony growth outside of inhibitory zone.

C. Narrow zone of inhibition - 5 millimeters with pin-point colonies and then uniform colonies outside of inhibitory zone.

D. No inhibition. Colonies all uniform in size.

Conclusions

It is obvious that if the medication is added at the same time that the plates are inoculated, no growth

will result while at the end of seventy-two hours, the medication does not seem to affect the growth of the organism. It is probable that the seventy-two hour interval allows the organism ample opportunity to build up abundant growth and form copious spores that are not inhibited by the medicinal agent.

The best time for the addition of the medication would appear to be at the end of twenty-four hours. Addition of the medication at the end of forty-eight hours may distort the evaluation of the agent being tested.

It is possible that this relationship between the inoculation and the attempt to inhibit growth might have a bearing on the clinical activity of the fungicidal agents. While it may not be possible to add fungistatic or fungicidal agents to every exposed area, it does suggest that the prophylactic use of these agents might abort many cases of superficial fungous infection.

V - THE EFFECT OF pH ON THE ACTIVITY OF FUNGICIDAL AGENTS

In their original report, Peck and his associates (1938) stated: "the fungicidal and fungistatic effects of the fatty acids and their salts were not due to the hydrogen ion concentration produced by their addition to the media studied". They based their conclusion on an investigation which proved that Trichophyton species would grow equally well on Sabouraud's agar, irrespective of pH variations from 4.00 - 10.00.

Other workers, notably Foley and his associates (1947), have demonstrated certain fatty acids to be more fungicidal at pH 5 or 6 than at pH 7 or 8.

Since only controversial statements of this sort could be found in the literature, an investigation of the rôle of pH on the activity of fungicidal agents was undertaken.

Procedure

The problem of determining the pH of ointments is a difficult one to solve. Potentiometers, such as the Beckman, can be utilized, but the results obtained with these are subject to many factors, such as minute air spaces in the ointment, that tend to affect the result.

Since it was the purpose of this investigation to determine the relative rather than the exact degree of pH

of the preparations investigated, Nitrazine and Anachemia papers were used in the determinations of pH reported herein.

The following bases were used in this determination. All of them contained 5 per cent undecylenic acid incorporated into a washable-ointment base.

Base A. The preparation with just enough tartaric acid added to make it approximately pH 4.5. Tartaric acid was selected as the acidifying agent in preference to acetic, hydrochloric and other stronger acids that might possess some fungistatic or fungicidal activity in their own right.

Base B. Untreated base and having a pH of approximately 5.6.

Base C. The preparation with just enough 5 per cent potassium hydroxide solution added to make it approximately pH 7.8.

All of these bases were then evaluated by the method discussed in Part I of this investigation. The results of this determination were as follows:

Base	pH	Inhibition in Millimeters
A	4.5	8.6
B.	5.6	8.0
C.	7.8	7.4

Conclusions

These preliminary results indicate that there is a variation in the activity of fungicidal agents at different pH levels. The determinations which have been reported above, point to a more pronounced activity of these agents at a lower pH range.

The results of this limited study and the paucity of literature information now available, indicate the need for an extensive study on this subject. Such a study should encompass an evaluation of all of the fatty acids that are now in use when adjusted to various pH levels. This *in vitro* testing should also be carried out against a number of organisms rather than a single species. Only when such a determination is completed,will it be possible to set down dogmatic statements relative to the effect of pH levels on the *in vitro* activity of fungicidal agents.

VI - THE DETERMINATION OF THE DURATION OF FUNGICIDAL ACTIVITY

The question of "Just how long a fungistatic agent continues to exert its effect" is one that undoubtedly has occurred to many clinicians. The uncertainty of this fact in the minds of many physicians and dermatologists, is evidenced by the variety of directions that can be found on their prescriptions for agents used to combat superficial fungous infections. These directions may range from, "Apply on retiring" to, "Keep the area covered at all times with the medication".

While it was quite obvious that an *in vitro* evaluation of this factor would not necessarily parallel clinical reactions, a determination of the duration of activity of one of the newer agents, undecylenic acid, was undertaken.

Procedure

Because no previous reports concerning determinations of this nature were available, it was again necessary to improvise a method that would be practicable and yet scientific. After a number of trials and failures, the following method was devised.

To Sabouraud's agar plates that have been seeded in the usual fashion and incubated

for 24 hours, 1 cc. of a 5 per cent undecylenic acid ointment is added. The plates are then incubated at 20°C. Twenty-four hours after the addition of medication, one drop of a suspension of T. mentagrophytes is added within the zone of inhibition. This procedure is repeated at 24 hour intervals thereafter up to 96 hours. A wax crayon circle is made on the under-side of the plate to indicate where the inoculum was added. A drop of the same suspension is added to a control plate at the same time intervals to insure viability of the culture. Plates are examined daily for the presence of growth.

Results

The mold suspension added at the end of twenty-four and forty-eight hour intervals failed to grow while the seventy-two hour addition resulted in the growth of pin-point colonies. Suspensions added after seventy-two hours, ninety-six hours etc., resulted in uniform colony growth with no indication of inhibition.

Conclusions

It is apparent that the preparation studied (5 per cent undecylenic acid in Beeler Base) maintains full activity for at least forty-eight hours. Part of its

inhibitory activity is lost at the end of a seventy-two hour period and this preparation rapidly loses its efficacy from there on.

As mentioned before, these results cannot be translated into *in vivo* results. They do indicate, however, that other factors being equal, this preparation and possibly other similar compounds, should exert a clinical effect for a longer period than has been previously suspected.

GENERAL CONCLUSIONS

From the recorded observations that were made during the various investigative parts of this research, certain conclusions can be made at this time. They are as follows:

1. A new method for the *in vitro* evaluation of fungistatic or fungicidal agents has been developed. While certain techniques of this suggested method need more attention and refinement, it can be reported at this time that the method is of value. This is particularly true in the quantitative estimation of the activity of various fungistatic and fungicidal agents when incorporated in various vehicles.
2. The organism *T. mentagrophytes*, demonstrates some spore formation at the end of forty-eight hours and copious spore formation at the end of seventy-two hours when grown on Sabouraud's maltose agar or Honey Broth, U.S.P. XII, at 20°C.
3. The "washable-ointment" bases are superior to the "greasy" type of bases as vehicles for fungistatic or fungicidal agents when evaluated *in vitro* by the method described.
4. It has been demonstrated that in *in vitro*

evaluation of fungistatic and fungicidal agents, the agent to be tested should be added twenty-four hours after the plates have been seeded with the mold suspension. This suggests that the time of addition of medication in other *in vitro* evaluations should be studied.

5. The duration of fungistatic or fungicidal activity of one agent, undecylenic acid, 5 per cent in Beeler Base, is approximately seventy-two hours. A more definite time cannot be reported because the cultures were studied at twenty-four hour intervals only.
6. The pH of fungistatic or fungicidal preparations has a definite bearing on the effect of the preparation. The activity of one agent, 5 per cent undecylenic acid in Beeler Base, is more pronounced at a pH range or level on the acid side.

SECTION C

ABSTRACT AND BIBLIOGRAPHY

ABSTRACT

Fungous infections of humans were first demonstrated by Lagenbeck and Schoenlein in 1839. Seventy-one years later Sabouraud focused the attention of the medical world on this group of microorganisms with his publication Les Teignes (1910).

Two of the most common fungous infections afflicting mankind, "athlete's foot" and ringworm of the scalp, result from organisms belonging to the Fungi Imperfecti. Both of these conditions are literally "diseases due to civilization", since they are more prevalent in heavily populated areas.

Over the years, various types of medicaments have been used in the treatment of superficial fungous infections. These run the gamut from sulfur and vegetable drugs to the newer agents, such as the fatty acids.

Fungicidal agents should be able to penetrate to the location of the invading organism and kill the parasite without destroying or causing irritation to human tissue.

Various combinations of salicylic acid and other drugs have been extensively used to treat "athlete's foot". These combinations work by exerting a keratolytic effect. Other compounds such as phenolic derivatives, tincture of iodine and the organic mercurials have also been used in

treating this condition.

Underwood (1946) found from an extensive study, that many cases of overtreatment dermatitis were caused by the indiscriminate use of fungicidal preparations.

The introduction of the fatty acids as a safe and physiological approach in the treatment of "athlete's foot" has all but caused a renaissance in the therapy of this condition. The brilliant contributions of Peck and his associates (1938) laid the groundwork for the use of these agents in the treatment of fungous infections.

Many of the fatty acids that are now used in the treatment of fungous infections are normally found as constituents of sweat. Clinically, the various fatty acids have proved to be efficacious in the treatment of "athlete's foot".

Keeney found that a mixture of propionic acid and sodium propionate was particularly effective in a clinical study carried out at Johns Hopkins University (1944). He was motivated to evaluate the propionates due to their use as mold retarders in the dairy industry.

Undecylenic acid was also clinically evaluated in an extensive fashion. This agent was tested against a host of other preparations including propionic acid, sodium caprylate and many of the older fungicides. This investigation disclosed that undecylenic acid was the most

effective of the group evaluated.

Other workers have found that combinations of undecylenic acid and zinc undecylenate are superior to the acid alone.

Caprylic acid and sodium caprylate were thoroughly investigated from a clinical standpoint. Preliminary results indicate that these compounds will also have a place in the medical armamentarium.

Combinations of the various fatty acids and their salts have been suggested by Peck and Russ (1947). They hypothesize that since both propionic and caprylic acids are found in the sweat, the combination should be more effective than either of them separately.

Although clinical tests have not been extensive enough to demonstrate the striking superiority of any single fatty acid, as a group they are safer and more effective agents than other medicaments in the therapy of "athlete's foot".

Advances have also been made in the treatment of tinea capitis. While this condition may be due to either of two organisms, M. lanosum or M. audouini, infections caused by the latter organism are the most resistant to therapy.

Schwartz and his associates had ample opportunity to evaluate extensively a number of therapeutic agents

during an epidemic of scalp ringworm at Hagerstown, Maryland in 1944 and 1945. These workers found that salicylanilide and copper undecylenate, the copper salt of a fatty acid, were the most effective of the agents tested.

Other workers have suggested compounds of various kinds, many of them of their own formulation. Their suggested formulae do not, however, represent the safe and physiological therapeutic approach offered by the fatty acids and their salts.

An original investigation, instigated by a desire to develop a new method for the in vitro evaluation of fungicidal agents, resulted in several other interesting studies.

A proposed method for the evaluation of fungicidal agents is based on the Agar Plate technique. In this method, a suspension of T. mentagrophytes grown in Honey Broth U.S.P. XII is seeded in a uniform pattern on the surface of Sabouraud's maltose agar. Twenty-four hours later, 1 cc. of the agent to be tested is added to the center of the plate by means of a hypodermic syringe. Zones of inhibition are then measured in millimeters at subsequent twenty-four hour intervals.

The suggested method is of value in that the activity of an agent can be measured while it is in-

corporated in various bases. It does not involve a difficult technique and does not consume too much time.

Studies on the sporulation time of a culture of T. mentagrophytes proved that this organism forms copicus spores at the end of seventy-two hours when grown under the conditions described. This investigation was carried out both by studies with a dissecting microscope and by deep-well culture observations made under high-dry magnification.

Various ointment bases were evaluated by the new method. These included the greasy-type preparations and the new water-miscible type. When 5 per cent undecylenic acid was incorporated into these bases, the water-miscible type showed striking in vitro superiority. It is suggested that the greasy bases possibly mask the action of medicinal agents and do not liberate them as readily as the newer type of bases.

A factor that probably influences the results of in vitro evaluations is the time that the medicinal agent is added to the culture.

A series of tests in which the medication was added at various time intervals proved that the time at which the medication is added has a definite bearing on the results of in vitro tests. Clear-cut results are obtainable only when the medicinal agent is added twenty-

four hours after the plates are inoculated.

The controversial factor of the effect of pH on fungicidal activity was also investigated. Ointment bases containing one of the fatty acids were adjusted to various pH levels and evaluated by the suggested method. The results of these tests indicate that these agents are more effective when they are at a pH on the acid side. The need for a more extensive study of this factor is pointed out.

The duration of activity of fungicidal agents was also determined. This method of *in vitro* evaluation is described. Determinations of the duration of activity of one agent, 5 per cent undecylenic acid, by the suggested technique is reported. Five per cent undecylenic acid is effective for seventy-two hours and then rapidly loses its inhibitory activity.

The various investigations revealed many interesting factors concerned with the *in vitro* evaluations of fungicidal agents and their possible *in vivo* activity.

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